



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 47/48, C08G 65/32	A1	(11) International Publication Number: WO 98/18494 (43) International Publication Date: 7 May 1998 (07.05.98)
(21) International Application Number: PCT/US97/19257 (22) International Filing Date: 27 October 1997 (27.10.97) (30) Priority Data: 740,155 28 October 1996 (28.10.96) US (71) Applicant: MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US/US]; 77 Massachusetts Avenue, Cambridge, MA 02139 (US). (72) Inventors: MERRILL, Edward, W.; 90 Somerset Street, Belmont, MA 02178 (US). YEN, Diane, Rintzler; 27 Fiarmonst Street, Arlington, MA 02174 (US). SAGAR, Ambuj; 153 Richdale Avenue, Cambridge, MA 02140 (US). (74) Agents: PABST, Patrea, L. et al.; Arnall Golden & Gregory, LLP, 2800 One Atlantic Center, 1201 West Peachtree Street, Atlanta, GA 30309-3450 (US).		(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: METHODS FOR THE SYNTHESIS OF FUNCTIONALIZABLE POLY(ETHYLENE OXIDE) STAR MACROMOLECULES		
(57) Abstract <p>Methods are provided for the synthesis of poly(ethylene oxide) ("PEO") star macromolecules including functionalizable groups. In one embodiment, a core molecule including a plurality of dendritic branches or having a comb structure, and including a plurality of accessible reactive groups is reacted with functionalizable poly(ethylene oxide) ("PEO") molecules. The functionalizable poly(ethylene oxide) molecules include a reactive group capable of reacting with the reactive group on the core molecule, and a functionalizable group capable of being chemically modified, which optionally is protected. In the reaction, the PEO molecules are covalently attached to the core molecule, to form a PEO star macromolecule with terminal functionalizable groups. Preferably, the functionalizable PEO is a heterofunctional linear PEO which includes the reactive group at one terminus and the functionalizable group at the other terminus. The functionalizable groups on the PEO molecules then may be deprotected if necessary, and the further derivatized, for example, by the attachment of a biologically active molecule or polymer thereof.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

METHODS FOR THE SYNTHESIS OF FUNCTIONALIZABLE POLY(ETHYLENE OXIDE) STAR MACROMOLECULES

The present invention is generally in the area of the synthesis of poly(ethylene oxide) ("PEO") star molecules including reactive functionalizable groups.

Background of the Invention

Linear poly(ethylene oxide) (PEO) has been of interest for a long time due to its many unusual physical chemistry properties, including its ability to repel other polymers in aqueous solution, which leads to protein repulsion by both PEO in solution and by surfaces covered by immobilized PEO. Harris, J. M., *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications*; Plenum Press: New York, 1992. Recently, there has been increasing interest in PEO star molecules. PEO star molecules are a form of a branched polymer which include PEO "arms" attached to a central core. The core molecular weight is usually a small fraction (<6%) of the total molecular weight of the star molecule. PEO star molecules have been immobilized on surfaces in the form of hydrogel layers which are biocompatible and non-thrombogenic. U.S. Patent No. 5,275,838 and 5,171,264 to Merrill, the disclosures of which are incorporated herein by reference.

PEO star molecules have been prepared by a living anionic polymerization reaction using a divinylbenzene ("DVB") core. Lutz and Rempp, *Makromol. Chem.*, 189:1051 (1988); Gnanou *et al.*, *Makromol. Chemie*, 189:2885-2892 (1988); and Merrill, *J. Biomater. Sci. Polymer Edn.*, 5:1-11 (1993). The cores are synthesized first, followed by growing PEO arms outward from the core using ethylene oxide. This method produces a sample of PEO star molecules which are very polydisperse, possibly due to an uneven growth rate of the cores leading to star molecules with varying functionalities. The PEO star molecules

also have been found to be difficult to fractionate using conventional techniques such as ultrafiltration or classical temperature manipulation.

Van Genderen and Meijer (1st International Symposium on Polymer Therapeutics, January 10-12, 1996, University of Alabama School of Pharmacy) reported the attachment of methoxy terminated oligo(ethylene oxides) to a fifth generation poly(propylene imine) dendrimer. The resulting star-like molecule therefore was produced with unreactive terminal methoxy groups which could not readily be further derivatized, and with very short arms, including three ethylene oxide units per arm.

There is a need for the development of methods for the synthesis of monodisperse, well characterized PEO star molecules. There also is a need in many biomedical applications for the development of methods for the synthesis of PEO star molecules with PEO arms of significant length with functionalizable groups which are capable of reacting with one or more additional molecules, such as biologically active molecules.

It is therefore an object of the invention to provide PEO star molecules which are monodisperse. It is a further object of the invention to provide star molecules having as arms PEO chains of significant length. It is a further object of the invention to provide methods for the synthesis of PEO star molecules which include terminal functionalizable groups. It is another object of the invention to provide PEO star molecules with functionalizable groups which are capable of reacting with one or more additional, biologically active molecules such as therapeutic agents or specific binding molecules.

Summary of the Invention

Methods are provided for the synthesis of poly(ethylene oxide) ("PEO") star macromolecules including functionalizable groups. In one embodiment, a core molecule, such as a dendritic molecule including a plurality of dendritic branches, with terminal reactive groups ("first reactive groups"), is reacted with functionalizable poly(ethylene oxide)

("PEO") molecules. The functionalizable poly(ethylene oxide) molecules include a reactive group capable of reacting with the first reactive group on the dendritic molecule (a "second reactive group"), and a functionalizable group which is not reactive with the first reactive group(s) on the dendritic molecule (a "third reactive group") but which is capable of being chemically modified, and which optionally is protected. In the reaction, the PEO molecules are covalently attached to the termini of the branches on the dendritic molecule, to form a PEO star macromolecule with terminal functionalizable (or "third reactive") groups. Preferably, the functionalizable PEO is a heterofunctional linear PEO which includes the reactive group (or the "second reactive group") at one terminus and the functionalizable group (or the "third reactive group") at the other terminus. The functionalizable groups on the PEO molecules then may be deprotected if necessary, and then further derivatized, for example, by the attachment of a biologically active molecule or polymer thereof.

Brief Description of the Drawings

Figure 1 is an illustration of a core molecule including a plurality of reactive groups, X.

Figure 2 is an illustration of a dendrimer core molecule including 64 terminal cyano groups.

Figure 3 is an illustration of a comb-like core molecule including a plurality of reactive groups, X.

Figure 4 is an illustration of a hyperbranched core molecule including a plurality of reactive groups, X.

Figure 5a illustrates the structure of a heterofunctional PEO molecule including reactive group, Y, and functionalizable group, Z.

Figure 5b illustrates the structure of a linear heterofunctional PEO molecule with a terminal N-succinimidyl succinate group.

Figure 5c illustrates the structure of a linear heterofunctional PEO molecule with a terminal N-succinimidyl propionate group.

Figure 5d illustrates the structure of the N-succinimidyl ester of carboxymethylated PEO.

Figure 5e illustrates the structure of a linear heterofunctional PEO molecule with a terminal N-succinimidyl carbonate group and a terminal methoxy group.

Figure 5f shows the structure of a linear heterofunctional PEO molecule including a terminal N-succinimidyl propionate group and a terminal t-boc blocked amino group.

Figure 5g shows the structure of a linear heterofunctional PEO including a terminal reactive group, N-succinimidyl propionate, and a terminal hydroxyl group.

Figure 6a shows the structure of a linear PEO including a terminal methoxy group and a terminal reactive group, Y.

Figure 6b shows the structure of a linear PEO molecule including the terminal reactive group, N-succinimidyl propionate, and a terminal methoxy group.

Figure 7 is an illustration of a PEO star macromolecule including eight PEO arms provided with a terminal functionalizable group, Z.

Figure 8 is an illustration of a PEO star macromolecule provided with PEO arms which include two types of terminal functionalizable groups, Z_1 and Z_2 .

Figure 9 is an illustration of a PEO star macromolecule including PEO arms of two different lengths, wherein the shorter arms include the terminal group Z_1 , and the longer arms include terminal group Z_2 .

Figure 10 is an illustration of a PEO star macromolecule including longer PEO arms terminated by an inert methyl group, and shorter PEO arms terminated by a functionalizable group, Z.

Figure 11 is an illustration of a PEO star macromolecule including plural PEO arms terminating in a hydrolysable moiety, M, linked to a functionalizable group, Z.

Figure 12 is a gel permeation chromatography ("GPC") chromatogram showing light scattering and refractive index signals versus

elution volume of the product of the reaction of a generation 4 poly(amidoamine) ("PAMAM") dendrimer with MEO-PEO-CH₂CH₂CONHS.

Figure 13 is a graph showing the logarithm of molecular weight (Mn) as a function of elution volume obtained from GPC of linear PEO and PEO star molecules, of which the PEO arm molecular weight was 5000.

Figure 14 is a table showing the calculated and experimentally determined molecular weights of PEO star macromolecules as a function of PEO type and dendrimer functionality.

Detailed Description of the Invention

Glossary of Terms

The following abbreviations are defined so that their use in this application is unambiguous:

15	<u>Abbreviation</u>	<u>Definition</u>
	PEO	linear poly(ethylene oxide)
	PEG	poly(ethylene glycol)
	STAR PEO	multi-armed molecule, with a compact central core and multiple PEO chains extending from the central core
20	NHS	N-hydroxysuccinimide
	t-boc	tert-butoxycarbonyl
	Mw	weight average molecular weight
	Mn	number average molecular weight
25	pdi	polydispersity index (Mw/Mn)

Methods for the synthesis of substantially monodisperse and functionalizable poly(ethylene oxide) ("PEO") star macromolecules are provided. The functionalizable PEO star macromolecules advantageously may be readily further derivatized, for example by the attachment of

therapeutically active biological molecules, diagnostic agents, targeting agents, or combinations thereof.

Formation of Functionalizable PEO Star Macromolecules

Functionalizable PEO star molecules are synthesized by attachment
5 of heterofunctional PEO chains or "arms" to a core molecule including a plurality of reactive groups which is shown schematically in Figure 1. For example, the core molecule may be a dendritic branched molecule including reactive groups at the termini of the branches which are capable of reacting with a functional group on the heterofunctional PEO chains.
10 An example of dendritic branched core molecule is shown in Figure 2. Alternative core molecules include comb-like molecules carrying a reactive side group, X, such as poly(amino styrene), illustrated in Figure 3, wherein the degree of polymerization may be, for example, in the range of about 10 to 500. In another embodiment, as illustrated in Figure
15 4, the core molecule can be a hyperbranched molecule including reactive groups, X, such as amino groups.

In general, the core molecule should be multifunctional. Preferably, the core molecule is compact, in that, in the resulting star macromolecule, the core is the center of the star molecule; the core has a
20 small volume compared with the volume of the star molecule in solution; and the core is effectively shielded by the PEO arms of the star molecule in solution against contact with large biologically active molecules, such as proteins. While the core molecule preferably is compact, it may nonetheless have interior void space capable of holding small molecules,
25 such as described by E.W. Meier *et al.* (*Polym. Mater. Sci. Eng.*, 73, 123 (1995)), and illustrated in Figure 2.

To form the PEO star macromolecule, a plurifunctional core molecule, $D-(X)_f$, where f is the number of reactive groups (X), is reacted with PEO chains or "arms" which preferably are in the form a linear
30 molecule, Z-PEO-Y, where the terminal group Y is capable of reacting with -X to form a covalent bond linking the PEO arm to the core. Preferably, the other terminal group Z is inert (for example due to the

presence of a protecting group) in that under the conditions of coupling - Y with -X, Z- cannot react with -X. After attachment of the PEO arms to the core to form the PEO star macromolecule, the Z group at the ends of the star molecule may if necessary be transformed, for example by a
5 deprotection step, to a group Z* which is reactive, allowing attachment of bioactive or other molecules, or further extension of arm length by addition of further molecules of Z-PEO-Y.

Suitable reactive groups or protecting groups which can be used in the reaction can be selected and designed using synthetic organic methods
10 available in the art, such as are described in Greene and Wuts, "Protective Groups in Organic Synthesis", Second Edition, John Wiley and Sons, Inc., New York, 1991; and in March, "Advanced Organic Chemistry, Reactions, Mechanisms, and Structure", John Wiley & Sons, New York, 1992.

15 For example, linear PEO molecules having an N-hydroxysuccinimidyl ester functional group at one end are reacted with a core molecule, such as a dendritic molecule having a predetermined number of primary amine groups. The other end of the PEO molecule includes a terminal functional group capable of being further reacted, but
20 not capable of reacting with the primary amine groups, for example, a hydroxyl or t-boc protected amine group. The reaction produces PEO star macromolecules including a central core attached to the PEO arms via an amide linkage. Depending on the terminal functional group on the linear PEO, the outer ends of the star molecules will be capable of further
25 reaction, after deprotection if necessary. For example, a t-boc protecting group can be removed from a terminal amine group by adding a dilute solution of hydrochloric acid. In this latter case, star molecules terminating with primary amines can be further extended by reacting the terminal amine groups with additional PEO chains or with other
30 molecules.

PEO star macromolecules with a large number of PEO arms, for example, 150 or more arms may be designed and synthesized using this

method. The star PEO macromolecules have more end groups than their linear counterparts of equivalent molecular weight, therefore providing a greater number of points of attachment for other molecules, for example, bioactive molecules such as enzymes, peptides, and organic drug molecules or diagnostic or targeting agents, or for attachment to surfaces upon which PEO is to be immobilized. Even using PEO chains of molecular weight measured in thousands (for example, 5,000 - 10,000), it is possible to attach preformed arms in large number to preformed cores, and to create substantially monodisperse star macromolecules having a low polydispersity index, for example, less than about 1.15.

Core Molecules

The star molecules are synthesized by reaction of heterofunctional PEO chains with a core molecule which is a plurifunctional molecule $D-(X)_f$, where f is the number of reactive groups X per molecule. In one preferred embodiment, the core molecule is a dendrimer including a plurality of dendritic branches with terminal reactive groups. As used herein, the term "dendrimer" refers to a molecule built up from a single starting molecule by sequential covalent reactions with a molecule having reactive sites to produce a branched molecule including terminal reactive groups. An example of the synthesis of a dendrimer is the synthesis of poly(amido-amine) ("PAMAM") StarburstTM dendrimers including terminal amine groups, as described in Tomalia *et al.*, *Macromolecules*, 19 2466 (1986); and U.S. Patent No. 4,568,737 to Tomalia *et al.*, the disclosures of which is incorporated herein. Each sequential reaction builds the dendrimer up generation by generation, wherein after each reaction, the core has twice the number of active sites (amine groups) on its surface as the generation before. For example, ethylenediamine may be used as the starting material, and reacted in alternate reactions with methyl acrylate and ethylene diamine. One advantage of dendrimers is that they may be synthesized in successive generations to have a precise, known functionality. For example dendrimers may be synthesized with 4, 8, 16, 32, 64, 128, 256, or more primary amine groups. PAMAM

dendrimers manufactured by Dendritech, Inc., are available commercially in several generations (sizes) from Aldrich Chemical Company, St. Louis, MO, as well as from Dendritech, Midland, MI. These primary amine groups may be used as the point of attachment for the PEO chains to be
5 the "arms" of the star molecule.

Preferably the dendrimer is a dense, branched, spherical molecule, and has between about 8 and 256 reactive groups. Figure 2 shows a dendrimer which has 64 cyano (CN) groups that can be hydrogenated to become 64 amino groups. In a preferred embodiment, the dendrimer is
10 built up symmetrically generation by generation to produce a symmetrically branched structure including a plurality of terminal reactive groups. However, other possible structures including terminal reactive groups may be used as the core molecule including comb structures, as illustrated in Figure 3, and hyperbranched molecules, as illustrated in
15 Figure 4.

In addition to amines, other reactive groups which may be present on the core include carboxylic acids and modified carboxylic acid derivatives such as acid chlorides. Exemplary dendrimers include amine terminated polyamidoamine dendrimers, poly(propylenimine) dendrimers,
20 and polylysine dendrimers.

Dendrimers with reactive terminal groups can be prepared using techniques described, for example, in O'Sullivan, C&EN, pp. 20, August 16, 1993; and Haggin et al., C&EN, pp 26-27, Feb. 6, 1995. Other branched molecules with reactive groups which can be used as the PEO
25 star core include cascade branched globular poly(α,ϵ -L-Lysine) macromolecules, described in Aharoni *et al.*, *Macromolecules*, 15: 1093-1098 (1982); and in U.S. Patent No. 4,289,272 to Denkwalter, the disclosures of which is incorporated herein.

Heterofunctional Poly(Ethylene Oxide) Molecules

30 The PEO star macromolecules preferably are formed by attachment of heterofunctional PEO molecules to the branched core molecule including terminal reactive groups, such as a dendrimer core.

As used herein, the term "heterofunctional PEO molecule" refers to PEO molecules, or poly(ethylene glycol) molecules which are preferably linear, and which are provided with different terminal groups at each end of the linear molecule, wherein one terminal group is a reactive group, Y,
5 capable of covalent reaction with the reactive group, X, on the core molecule; and wherein the other terminal group, Z, is a functionalizable group which is capable of being chemically modified, after attachment of the PEO molecules to the core. In a preferred embodiment, the linear heterofunctional PEO molecules include between about 10 to 350 ethylene
10 oxide units per chain.

The heterofunctional PEOs are defined in one embodiment, as illustrated in Figure 5a, as linear molecules having the following formula:



wherein n is between about 10 and 500, preferably between about
15 50 to 250;

wherein Y is a reactive group capable of covalent reaction with the reactive group, X, on the core molecule; and

Z is a functionalizable group which is capable of being chemically modified after the covalent reaction.

20 The reactive group Y on the heterofunctional molecule will depend on the reactive group present on the core molecule, and can be, for example, -OH, NH₂, carboxylic acid, a modified carboxylic acid such as an ester, an isocyanate or an aldehyde. In the case where the reactive group X on the core molecule is an amine, the reactive group Y can
25 include a functional group capable of forming covalent bonds with a free amine.

In one embodiment, the reactive group may be, for example, an N-hydroxy succinimidyl ("NHS") derivative, such as an N-succinimidyl succinate, as illustrated in Figure 5b; an N-succinimidyl propionate, as
30 illustrated in Figure 5c; an N-succinimidyl ester of a carboxymethyl

group, as illustrated in Figure 5d; or an N-succinimidyl carbonate, as illustrated in Figure 5e. The functionalizable group, Z, on the heterofunctional PEO molecule may include groups such as a hydroxyl or protected hydroxyl group, a carboxyl or a protected carboxyl group, or an amino or protected amino group. Figures 5f and 5g illustrate heterofunctional PEO molecules in which the NHS derivative is the N-succinimidyl succinate and the functionalizable end Z is respectively a t-boc blocked amino group and a hydroxyl group.

Other heterofunctional PEOs may be designed and synthesized using organic chemistry techniques available in the art, and/or may be obtained commercially. Linear PEOs (which are also referred to as PEGs) with modified end groups are available commercially from Shearwater Polymers, Inc. (Huntsville, AL) which have terminal groups including epoxide, hydroxyl, carboxyl, amine, t-Boc protected amine, and various N-hydroxyl succinimidyl esters. Figures 6a and 6b illustrate linear PEO molecules provided with a terminal methoxy group and a terminal reactive group, Y and N-succinimidyl propionate, respectively.

Derivatization of the Star Macromolecules

The PEO star macromolecules in one embodiment, as illustrated schematically in Figure 7, include plural PEO chains provided with a terminal functionalizable group, Z. The PEO chains in the star molecule may be derivatized, for example, by attachment of a biomolecule or polymer thereof to free hydroxyl or other functionalizable group, Z, at the termini of the PEO chains. Preferred functionalizable groups Z on the PEO chains are protected $-NH_2$ and $-OH$. Biologically active molecules, such as antibodies, enzymes, growth factors, or diagnostic agents may be attached to the PEO star molecules, after the functionalizable group Z has become active by deprotection, or directly (e.g. $Z = -OH$).

Alternatively, two or more different molecules may be attached to the star molecule.

Any of a range of biological molecules, such as nucleic acids, amino acids, saccharides or polymers thereof may be attached to the

terminal reactive groups on the PEO chains of the star molecules. As used herein, the term "nucleic acids, amino acids and saccharides, or polymers" includes natural and synthetic derivatives thereof. Exemplary biological molecules include proteins such as enzymes, antibodies and cell recognition molecules. Other exemplary molecules include cell adhesion molecules, growth factors, metals, lipids, and polysaccharides, as well as organic drug molecules. As used herein, the term "protein" is defined as a polymer of two or more amino acids or amino acid derivatives, such as a peptide, polypeptide or enzyme. The biological molecule attached to the PEO star molecule also may be a specific binding molecule, such as a monoclonal antibody or fragment thereof, or equivalent thereof. The biological molecule also may be a member of a specific binding pair such as antigen/antibody or ligand/receptor.

In a further embodiment, at least two different biological molecules or polymers thereof may be covalently attached to the functionalizable groups on the PEO chains, to produce a multifunctional star molecule. For example, two different enzymes, or an enzyme and a polysaccharide may be attached to the PEO star molecule. In one embodiment, two different functionalizable groups, Z_1 and Z_2 , may be provided on the PEO star macromolecule for further reaction, as illustrated in Figure 8.

In a further embodiment, illustrated in Figure 9, the heterofunctional PEO molecules are provided in the reaction in two or more different length fractions, ranging, for example, from about 10 to 350 ethylene oxide units, wherein the fractions of different lengths have a narrow MW distribution, and may include the same or different functionalizable groups or reactive groups. The PEO molecules of different lengths and functionalizable groups may be reacted with the dendrimer to produce a PEO star macromolecule with a preselected distribution of PEOs of selected lengths and a selected distribution of terminal functionalizable groups. If desired, nonfunctionalizable PEO molecules, including nonreactive groups such as terminal methyl groups,

also may be incorporated into the PEO star macromolecule, as illustrated in Figure 10.

In a further embodiment, as illustrated in Figure 11, the functionalizable groups, Z, on the linear heterofunctional PEO molecules may be linked to a hydrolyzable moiety, M, either before or after formation of the PEO star molecule, and the hydrolyzable moiety further may be linked to a biologically active molecule. Exemplary hydrolyzable groups include lactic acid, glycolic acid, poly(lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid), and polyanhydrides. PEO star molecules useful in drug delivery applications may be fabricated with a proportion of shorter PEO chains attached via the functionalizable group to a hydrolyzable moiety linked to a biologically active molecule, and a proportion of longer PEO molecules on the star molecule attached to a cell recognition molecule.

Hydroxyl termini of PEO chains on the star molecules can be activated and then a ligand, such as an antibody, may be attached to the termini, as described in U.S. Patent Nos. 5,171,264 and 5,275,838, the disclosures of which are incorporated herein by reference. For example, the free hydroxyl groups can be activated with tresyl chloride and then reacted with a ligand that contains a free amino acid or thiol group to form a covalent linkage with the ligand. Harris, *J. Macromolecular Sci. Reviews in Macro. Chem. Phys.*, C25(3):325-373 (1985).

The present invention will be further understood by reference to the following non-limiting examples, in which the following materials and equipment were used.

Equipment and Materials

Analysis of all samples was done using gel permeation chromatography (GPC), in series with refractive index (RI) and light scattering (LS) measurements. Determination of the molecular weight distribution and polydispersity of all samples was made using gel permeation chromatography (GPC) in conjunction with light scattering (LS). The GPC used was a Waters Model 150C containing two Tosohaas

TSK-gel columns in series, G6000PW and G4000PW. The GPC eluate from the columns pass through a Wyatt Dawn Model F laser photometer and then through the refractive index detector contained within the Model 150C system. Voltage measurements taken from the detectors are
5 recorded every second and converted to light intensity and refractive index measurements respectively. The differential refractive index increment for the PEO stars is assumed to be equal to that of linear PEO, since the weight fraction of PEO is greater than 0.95. All calculations were made through ASTRA, a software package designed for use with the
10 Wyatt Dawn Model F, which is run on a CUI 386 PC. Polydispersity is measured as M_w/M_n , with a polydispersity index (pdi) of 1.15 or less taken as indicating that the sample is substantially monodisperse.

The heterofunctional linear PEO molecules were obtained from Shearwater Polymers, Inc., Huntsville, AL. The dendrimers used as
15 cores of the PEO star molecules were PAMAM StarburstTM dendrimers provided and synthesized by Dendritech, and are synthesized as described in Tomalia *et al.*, *Macromolecules*, 19:2466 (1986), with the exception that ethylene diamine was used as the starting molecule instead of ammonia, followed by alternate reactions with methyl acrylate and
20 ethylene diamine. These dendrimers were synthesized in successive generations to have 16, 32, 64, 128, or 256 primary amine groups.

Example 1: Reaction of Dendrimers with Heterofunctional Poly(Ethylene Glycol) Molecules.

Different PEO star macromolecules were synthesized by the
25 reaction of PAMAM dendrimers with 16, 32, 64, 128, or 256 primary amine groups with the heterofunctional PEO molecules, MeO-PEO-CH₂CH₂CONHS (MW 5000), HO-PEO-CH₂CH₂CONHS (MW 4000), and tboc-HN-PEO-CH₂CH₂CONHS (MW 3400). The molecular weights of the heterofunctional PEO molecules are those reported by the
30 manufacturer.

Reaction of Dendrimers with Heterofunctional PEO Molecules.

A known quantity of dendrimer was dissolved in 0.1 M sodium bicarbonate buffer. It was assumed that one heterofunctional PEO molecule would react with each primary amine group on the dendrimer's surface. Based on this assumption the dendrimer solution was then added to a 1.6x molar excess of heterofunctional PEO. The dendrimer was added to the buffer before dissolving the heterofunctional PEO in buffer, due to the high rate of hydrolysis of the NHS group and the desire to avoid contact of the PEO with water until the dendrimer was present. The solution was then stirred for 2 hours to permit attachment of the PEO molecules to the dendrimer to form star PEO arms covalently linked to the dendrimer.

Terminal t-boc protecting groups on the PEO star molecule if present were removed by treatment with dilute hydrochloric acid and stirring overnight. This yielded a PEO star molecule with free terminal amino groups, capable of binding or reacting with further molecules.

Separation of Unreacted Heterofunctional Poly(Ethylene Oxide)

The PEO star macromolecules prepared in the above manner contained excess unreacted linear heterofunctional PEO. This PEO was removed via ultrafiltration using a stirred ultrafiltration cell (Amicon Corp., Beverly, MA). The membranes used depended on the size of the star synthesized. For example, a star synthesized by reacting a dendrimer containing 64 amine groups with methoxy PEO (MW=5000), is completely retained by an Amicon YM100 (100,000 molecular weight cutoff (MWCO), regenerated cellulose) membrane. Therefore this membrane was used to separate these stars from the methoxy PEO. However, a star prepared using a dendrimer containing only 16 amino groups would pass through a YM100 membrane. Therefore a YM30 (30,000 MWCO, regenerated cellulose) membrane was used to separate this star from linear PEO. The presence of unreacted PEO remaining in solution was monitored by injecting a sample of both the permeate (solution passing through the membrane) and the retentate (solution

retained by the membrane) into the GPC/LS. Stars diafiltered into ion free water from buffer solution were then filtered using a 0.5 micron filter and the water was lyophilized off.

Synthesis of PEO Star Molecules by Reaction of PAMAM

5 Dendrimers with MeO-PEO-CH₂CH₂CONHS, HO-PEO-CH₂CH₂CONHS, and t-Boc-PEO-CH₂CH₂CONHS

Eleven different types of star molecules were synthesized. Three types of heterofunctional PEO molecules, i) MeO-PEO-CH₂CH₂CONHS (MW 5000), shown in Figure 6b; ii) HO-PEO-CH₂CH₂CONHS (MW
10 4000), shown in Figure 5g; and iii) t-Boc-PEO-CH₂CH₂CONHS, shown in Figure 5f, were reacted with PAMAM dendrimers of generation 2-6, having functionalities ranging from 16 to 256.

A sample GPC chromatogram taken after reacting a generation 4 dendrimer containing 64 primary amine groups with MeO-PEO-
15 CH₂CH₂CONHS is shown in Figure 12. The first peak shows the star molecule produced. The second peak is unreacted linear PEO, while the last peaks are due to salt. The narrowness of the first peak and the near overlap of the refractive index trace with the light scattering trace indicate that a nearly monodisperse sample was produced. The number average
20 molecular weights (M_n), polydispersity indexes (pdi - weight divided by number average molecular weight) and functionalities for the star macromolecules are summarized in Table 1 in Figure 14. These results show that when reacted with dendrimers of low functionality (<32), the heterofunctional PEOs react with substantially all the amine groups to
25 create a relatively monodisperse molecule. However, as the functionality gets to be greater than 32, steric hindrances preclude all the PEOs from being able to react with all the amine groups on the dendrimers and M_n is less than expected, although star molecules are produced with up to 140 arms. Advantageously, even when not all the amine groups on the
30 dendrimer are reacted with PEO, a nearly monodisperse sample of star molecules still results.

Figure 13 shows a plot of log Mn as function of elution volume for linear PEO molecules and PEO star molecules formed from HO-PEO-CH₂CH₂CONHS of 5000 molecular weight. At the same elution volume, the star molecule has a much higher molecular weight than the linear PEO molecule. For example, the Mn of the star molecule prepared from HO-PEO-CH₂CH₂CONHS eluting at 7 ml is 324,000. The molecular weight of the linear PEO eluting at the same volume is only 94,000, thus confirming that a PEO star molecule has a considerably smaller hydrodynamic volume than a linear PEO molecule of equal molecular weight.

Example 2: Synthesis of PEO Star Molecules by Reaction of Dendrimers with a Heterofunctional PEO including a Protected Amine Group.

Using the general procedure described above, a generation 2 PAMAM dendrimer, with an amine functionality ("f") of 16 was reacted with the heterofunctional PEO, *t*boc-HN-PEO-CH₂CH₂CONHS of MW 3400. GPC/LS analysis indicated that the product PEO star molecule had a Mn of 88,100, and a pdi of 1.06. The same reaction using the generation 3 (f=32) dendrimer by GPC/LS analysis produced a PEO star molecule with a Mn of 173,000, and a pdi of 1.09. Reaction of the heterofunctional PEO with the generation 4 dendrimer (f=64) from GPC/LS analysis had a Mn of 239,000, and a pdi of 1.03.

Example 3: Preparation of PEO Star Molecules with Extended PEO Arms.

PEO star molecules with extended PEO arms may be synthesized using the methods described in Example 1. In some applications, it is desired to have long PEO arms on the star molecule. However, as the length of the arms increases, steric hindrance may prevent the reaction from reaching completion. To avoid this problem, a series of shorter chain PEO chains are sequentially reacted with the dendrimer, wherein the shorter chains include a terminal first group capable of reacting with free amines, and a second terminal protected amine group. After

attachment of the shorter PEO chains and deprotection of the amino group, the PEO can again be reacted with the star molecule, and the process can be repeated to grow PEO arms of a desired length.

In one example, eight microliters of generation 3 PAMAM
5 dendrimer was charged to 0.4 ml sodium bicarbonate buffer. This solution then was added to 33.8 mg of a heterofunctional linear PEO, tboc-HN-PEO-CH₂CH₂CONHS (3400 MW). Another 0.1 ml of buffer was used to rinse out the vial. After letting the reaction proceed overnight under constant stirring, a sample was taken for GPC/LS
10 analysis, from which Mn was determined to be 159,000, and the polydispersity index ("pdi") was found to be 1.16. The remainder of the solution was brought to 0.5N HCl, and left stirring overnight. The reaction mixture then was diafiltered into .1 M NaHCO₃ solution using an Amicon YM 100 membrane, thereby removing unreacted PEO. 44.5 mg
15 of the linear PEO then was charged to the reaction mixture which was left stirring for 3 hours. Another sample was taken for GPC/LS analysis, and the remainder of the solution was again brought to 0.5N HCl. The Mn of this sample was found to be 326,000, and the pdi was found to be 1.13. The diafiltration process was repeated, and 36.6 mg of the linear PEO
20 was added, and the reaction was allowed to proceed for three hours with constant stirring. GPC/LS analysis showed that the Mn was 588,000, and the pdi was 1.11.

Example 4. Preparation of PEO Star Molecules with 2K and 20K PEO Arms

25 20k methoxy ended NHS-PEG was added to a generation 3 PAMAM dendrimer having 32 amino groups in a molar ratio of 6 PEG molecules to 1 amino group. The solvent used was dichloromethane with TEA added as an acid scavenger and methanol added to solubilize the dendrimer. An excess of 2k hydroxy ended NHS-PEG was then added.
30 Results of GPC/LS analysis of the final product showed it to have a number average molecular weight equal to 170,000 with a polydispersity index equal to 1.10.

Example 5. Preparation of PEO Star Molecules with 2K and 20K PEO Arms

The same procedure was followed as in Example 5, except a 12:1 ratio of 20k PEG to dendrimer amino groups was used. The final product had a number average molecular weight equal to 295,000 with a polydispersity index equal to 1.05.

Example 6. Preparation of PEO Star Molecules with 2K and 20K PEO Arms

The same procedure was followed as in Example 5, except an 18:1 ratio of 20k PEG to dendrimer amino groups was used. The final product had a number average molecular weight equal to 353,000 with a polydispersity index equal to 1.03.

Conclusions: Neglecting the molecular weight of the dendrimer core, the final molecular weight is given by the formula:

$$M_{\text{final}} = (32-X)20,000 + 2000 X$$

Where X is the number of dendrimer amino groups which attach 2,000 molecular weight PEG and 32-X is the number of dendrimer amino groups which attach 20,000 molecular weight PEG. Thus, the following table can be constructed:

Table 2: Analysis of Experiments 4-6

<u>Example</u>	<u>PEG/NH₂ ratio</u>	<u>M</u>	<u>32-X</u>	<u>X</u>
Example 4	6:1	170,000	6	26
Example 5	12:1	295,000	13	19
Example 6	18:1	353,000	16	16

Since the number of dendrimer amino groups which attached 20,000 molecular weight PEG, 32-X, is close to the PEG/NH₂ ratio, the coupling reaction of the NHS ester on PEG to the amino group on the dendrimer is very efficient. Consequently, it is possible to predetermine the ratio of the two types of PEG arms to be attached to the dendrimer.

We claim:

1. (Amended) A method for synthesizing a star macromolecule containing poly(ethylene oxide) moieties that contain a terminal reactive group, comprising:
 - i) providing a compact core molecule comprising a plurality of first reactive groups;
 - ii) providing linear heterofunctional poly(ethylene oxide) ("PEO") molecules, wherein each PEO molecule comprises:
 - first and second termini;
 - a second reactive group at the first terminus capable of reacting with the first reactive groups on the core molecule; and
 - a third reactive group at the second terminus which is not capable of reacting with the first reactive groups on the core molecule; and
 - iii) reacting the first reactive groups on the core molecule with the second reactive groups on the PEO molecules to covalently attach the PEO molecules to the core molecule.
2. The method of claim 1 wherein the core molecule has a structure selected from the group consisting of a hyperbranched structure and a comb structure.
3. The method of claim 1 wherein the core molecule is a dendritic molecule including a plurality of dendritic branches comprising a first reactive group at the terminus thereof.
4. The method of claim 3 wherein the dendritic molecule is selected from the group consisting of an amine terminated polyamidoamine dendrimer, a poly(propylenimine) dendrimer, and a polylysine dendrimer.
5. (Amended) The method of claim 1 wherein the PEO star macromolecule is substantially monodisperse.
6. (Amended) The method of claim 3 wherein the dendritic molecule comprises between about 8 and 256 reactive groups.

7. (Amended) The method of claim 1 wherein the first reactive group is an amine.
8. (Amended) The method of claim 1 wherein the first reactive group is selected from the group consisting of a carboxylic acid and a modified carboxylic acid, the second reactive group on the PEO molecule is an amino group, and the third reactive group on the PEO molecule is selected from the group consisting of a protected hydroxyl group, a protected amino group, and a protected carboxyl group.
9. (Amended) The method of claim 1 wherein the second reactive group on the PEO molecule is selected from the group consisting of a carboxylic acid, a modified carboxylic acid, an isocyanate and an aldehyde.
10. (Amended) The method of claim 7 wherein the second reactive group on the PEO molecule is an N-hydroxysuccinimidyl ester of a carboxylic acid and the third reactive group is a hydroxyl group.
11. (Amended) The method of claim 1 wherein the third reactive group is selected from the group consisting of amino, protected amino, hydroxyl, protected hydroxyl, carboxyl and protected carboxyl.
12. (Amended) The method of claim 1 wherein the PEO molecules provided in step ii) have a length of about 10 to 350 ethylene oxide units.
13. (Amended) The method of claim 1 wherein the third reactive groups on the PEO molecules provided in step ii) are protected, and wherein the method further comprises deprotecting the third reactive groups after step iii).
14. (Amended) The method of claim 13 further comprising covalently attaching the third reactive groups on the PEO molecules to a biologically active molecule or polymer thereof.
15. The method of claim 14 wherein the biologically active molecule is selected from the group consisting of an amino acid, a peptide, a protein, a nucleic acid, a lipid, an organic drug, a saccharide and a polysaccharide.

16. The method of claim 14 wherein the biologically active molecule is selected from the group consisting of a cell adhesion molecule, a growth factor, and a metal.

17. The method of claim 14 wherein the biologically active molecule is a specific binding molecule.

18. (Amended) The method of claim 13 further comprising covalently attaching at least two different molecules or polymers thereof to the third reactive groups on the PEO chains on the star macromolecule.

19. (Amended) The method of claim 1 wherein the heterofunctional PEO molecules provided in step ii) comprise a mixture of different lengths ranging from between about 10 and 350 ethylene oxide units.

20. The method of claim 19 wherein the PEO molecules comprise first and second fractions of different lengths and of narrow molecular weight distribution.

21. (Amended) The method of claim 20 wherein the PEO molecules in the first and second fractions comprise different third reactive groups; and

wherein the PEO molecules in the first and second fractions comprise the same second reactive group.

22. (Amended) The method of claim 20 further comprising, after step iii), reacting the third reactive groups on one of the PEO fractions on the PEO star macromolecule with linear PEO molecules comprising a fourth reactive group.

23. (Amended) The method of claim 1 further comprising providing in step ii) PEO molecules that do not contain a third reactive group and which have a length greater than the heterofunctional PEO molecules and have the same second reactive group as the heterofunctional PEO molecules, to incorporate PEO molecules that do not contain a third reactive group into the star macromolecule.

24. (Amended) The method of claim 23 further comprising, after step iii), attaching the third reactive group on the PEO star macromolecules to a biologically active molecule.

25. (Amended) The method of claim 1 further comprising linking the third reactive groups on the PEO star macromolecules to a hydrolyzable moiety.

26. The method of claim 25 further comprising linking the hydrolyzable moiety to a biologically active molecule.

27. The method of claim 26 wherein the hydrolyzable moiety is selected from the group consisting of lactic acid, glycolic acid, poly(lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid), and polyanhydride.

28. (Amended) The method of claim 20 further comprising linking the third reactive groups on the fraction of longer PEO molecules on the star molecule to a cell recognition molecule, and linking the fraction of shorter PEO molecules to a hydrolyzable moiety linked to a biologically active molecule.

29. (Amended) A star macromolecule formed by:

i) providing a compact core molecule comprising a plurality of first reactive groups;

ii) providing linear heterofunctional poly(ethylene oxide) ("PEO") molecules, wherein each PEO molecule comprises:

first and second termini;

a second reactive group at the first terminus capable of reacting with the first reactive groups on the core molecule; and

a third reactive group at the second terminus; and

iii) reacting the first reactive groups on the core molecule with the second reactive groups on the PEO molecules to covalently attach the PEO molecules to the core molecule.

30. The star macromolecule of claim 29 wherein the core molecule has a structure selected from the group consisting of a hyperbranched structure and a comb structure.

31. The star macromolecule of claim 29 wherein the core molecule is a dendritic molecule including a plurality of dendritic branches comprising a first reactive group at the terminus thereof.

32. The star macromolecule of claim 29 wherein the PEO star macromolecule is substantially monodisperse.

33. The star macromolecule of claim 29 wherein the dendritic molecule is selected from the group consisting of an amine terminated polyamidoamine dendrimer, a poly(propyleneimine) dendrimer, and a polylysine dendrimer.

34. The star macromolecule of claim 29 wherein the dendritic molecule comprises between about 8 and 256 reactive groups, and wherein the PEO molecules have a length of between about 10 and 350 ethylene oxide units.

35. The star macromolecule of claim 29 further comprising at least one biologically active molecule or polymer thereof covalently attached to the third reactive groups on the PEO molecules.

36. The star macromolecule of claim 35 wherein the biologically active molecule is selected from the group consisting of an amino acid, a peptide, a protein, a nucleic acid, a lipid, an organic drug, a saccharide and a polysaccharide.

37. The star macromolecule of claim 29 wherein the heterofunctional PEO molecules provided in step ii) comprise first and second fractions of different lengths and of narrow molecular weight distribution, and include between about 10 and 350 ethylene oxide units.

38. The star macromolecule of claim 37 wherein the PEO molecules in the first and second fractions comprise different third reactive groups.

39. The star macromolecule of claim 37 further comprising a cell recognition molecule linked to the third reactive group on the fraction

of longer PEO molecules on the star macromolecule, and a biologically active molecule linked to a hydrolyzable moiety linked to the fraction of shorter PEO molecules on the star macromolecule.

40. A poly(ethylene) oxide ("PEO") star macromolecule formed by

i) providing a compact core molecule including a plurality of accessible first reactive groups;

ii) providing a mixture of linear heterofunctional and monofunctional PEO molecules, wherein the PEO molecules comprise a second reactive group capable of reacting with the first reactive group on the core, and wherein the linear heterofunctional PEO molecules comprise first and second termini wherein the second reactive group is on the first terminus and a third reactive group is present on the second terminus, and

iii) reacting the first reactive groups on the core molecule with the second reactive groups on the PEO molecules to covalently attach the PEO molecules to the core molecule.

41. The star macromolecule of claim 40 wherein the monofunctional PEO molecules have a substantially different degree of polymerization than the heterofunctional PEO molecules.

42. The star macromolecule of claim 40 further comprising a biologically active molecule attached to the third reactive group on the PEO star macromolecule.

1/5

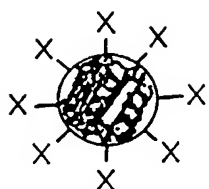


FIG. 1

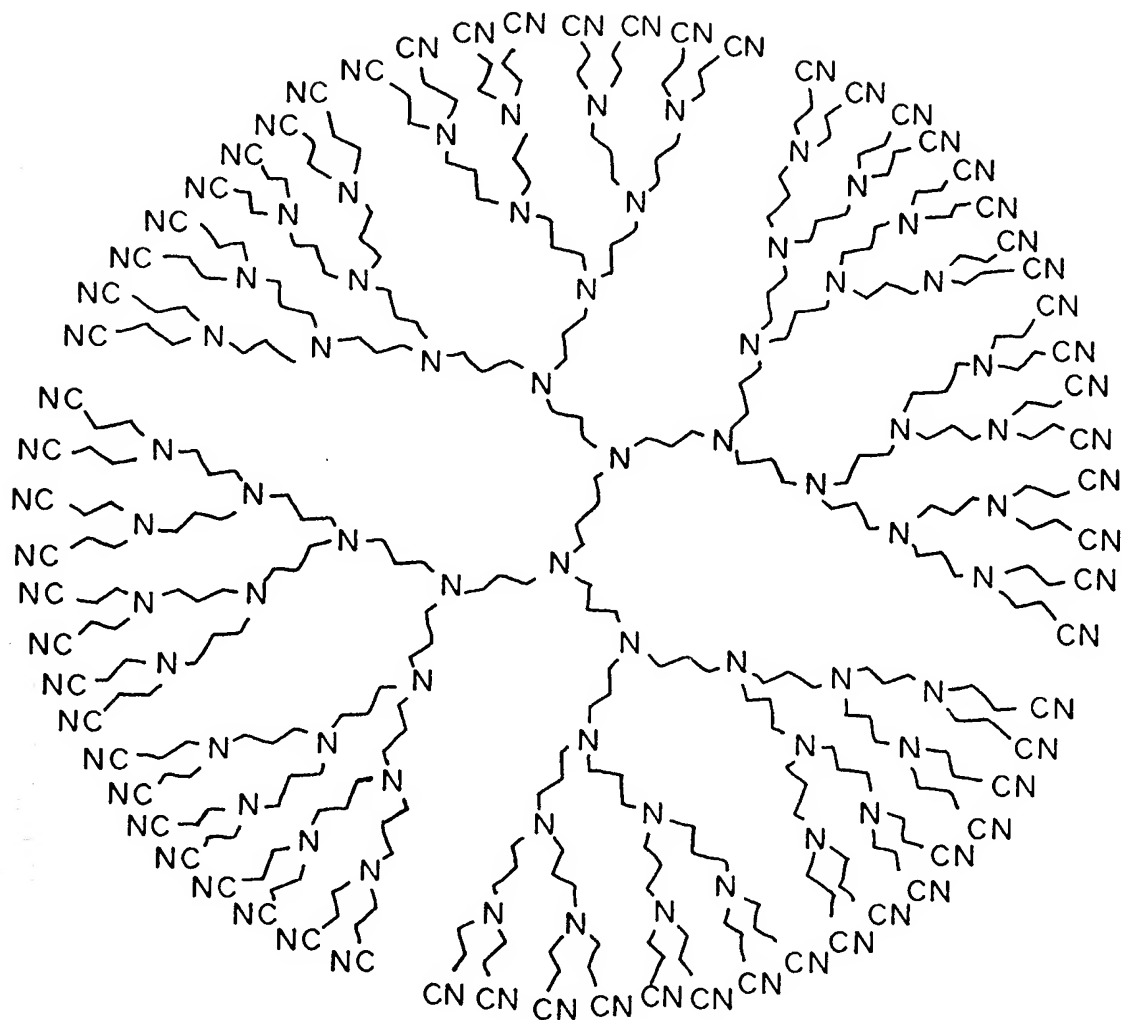


FIG. 2

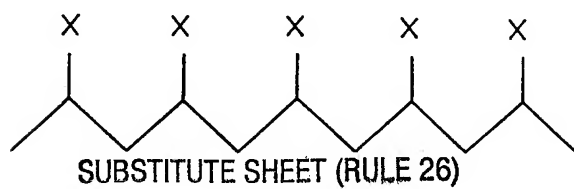
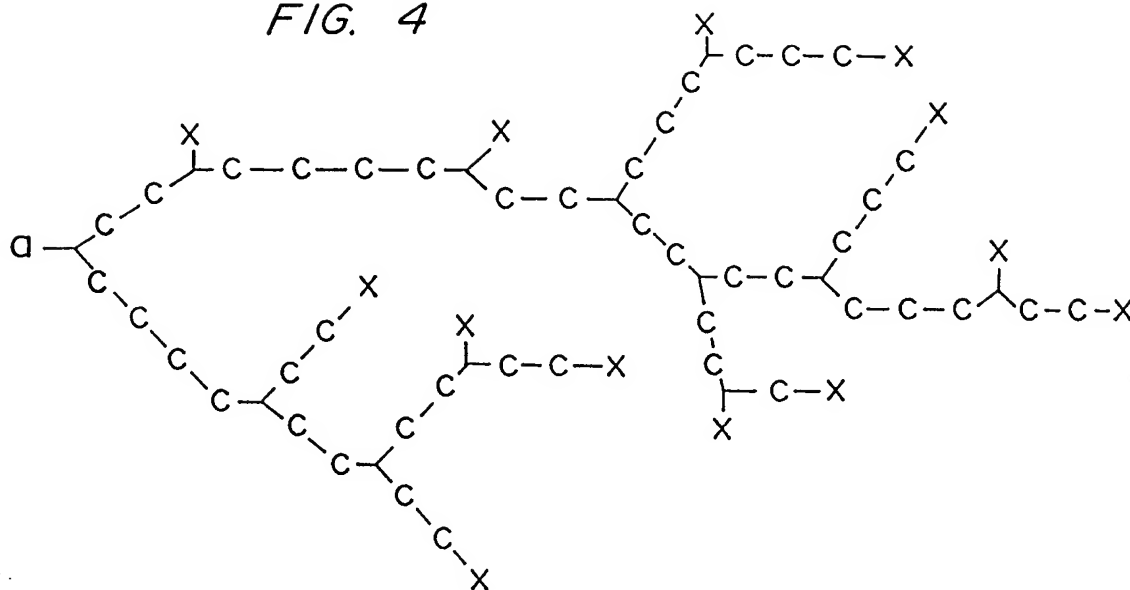
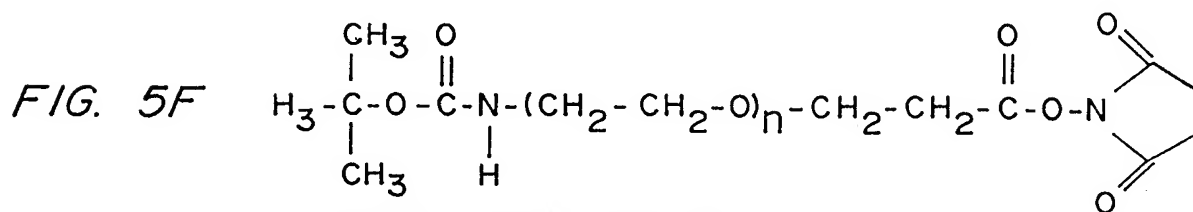
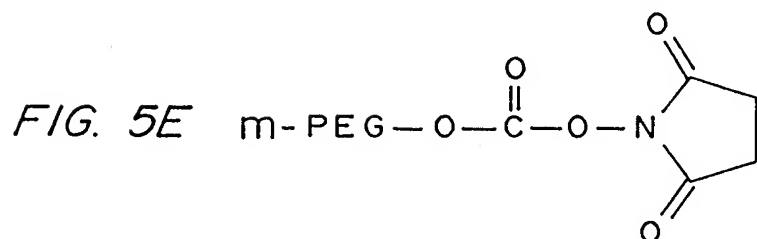
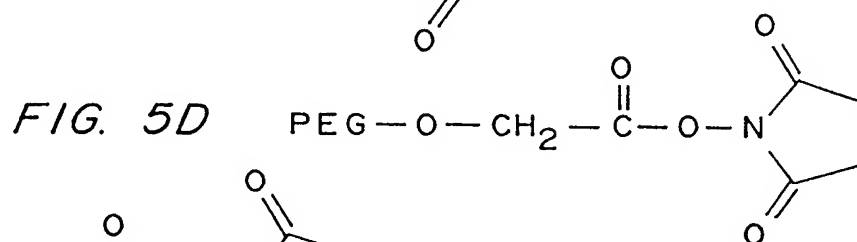
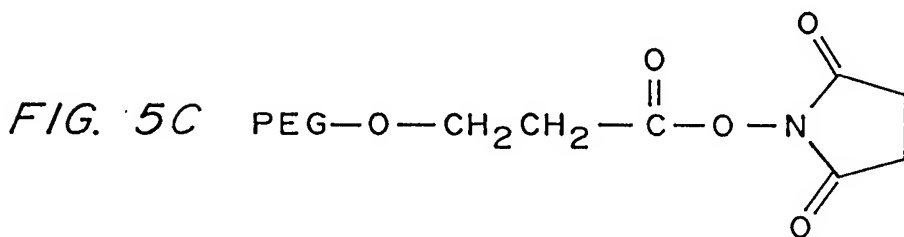
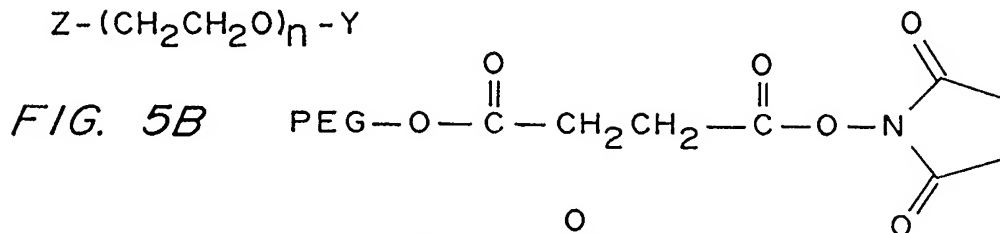


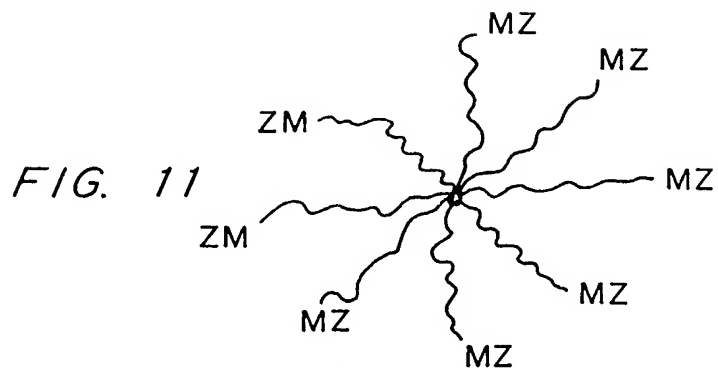
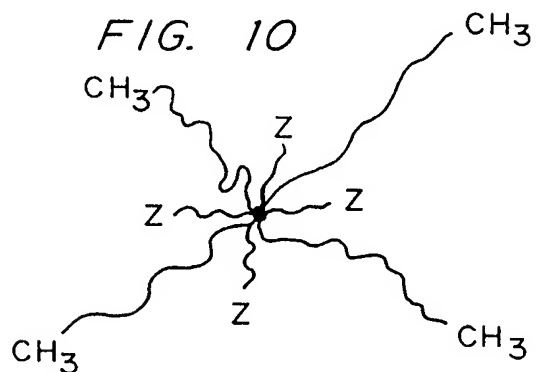
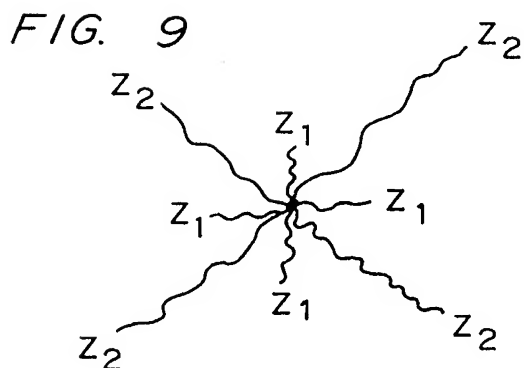
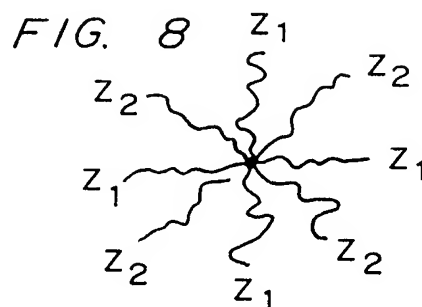
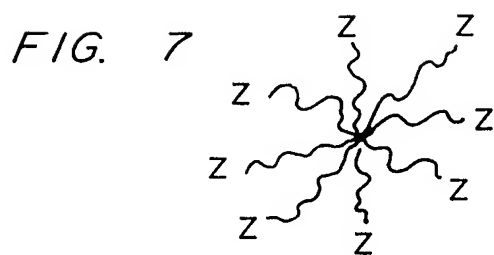
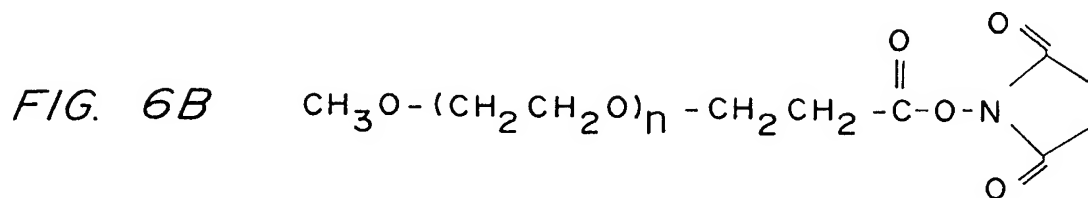
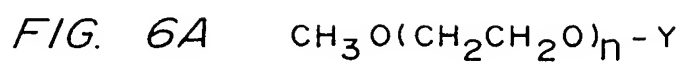
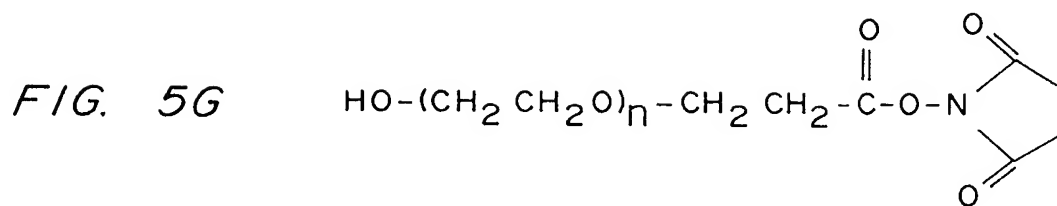
FIG. 3

2/5

FIG. 4

FIG. 5A $Z-(CH_2CH_2O)_n-Y$ 

3/5



SUBSTITUTE SHEET (RULE 26)

4/5

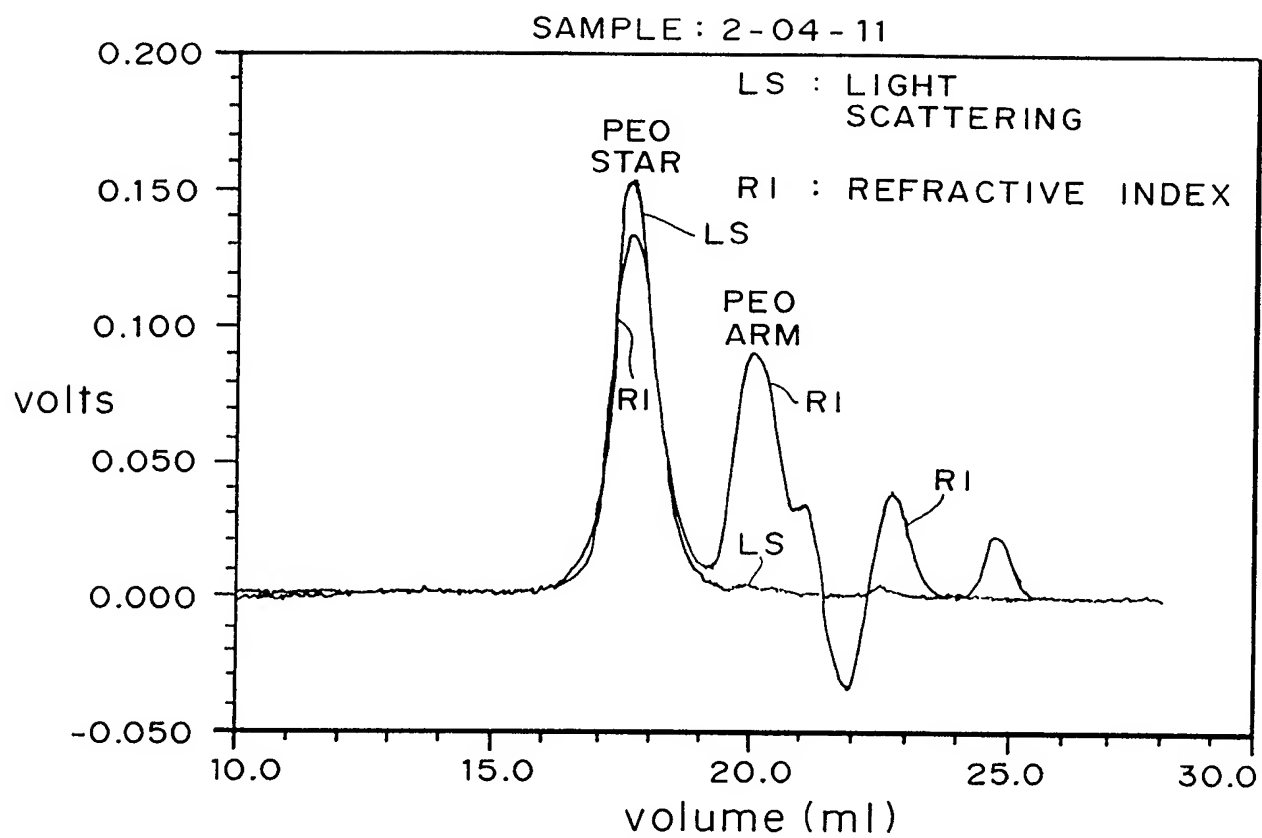


FIG. 12

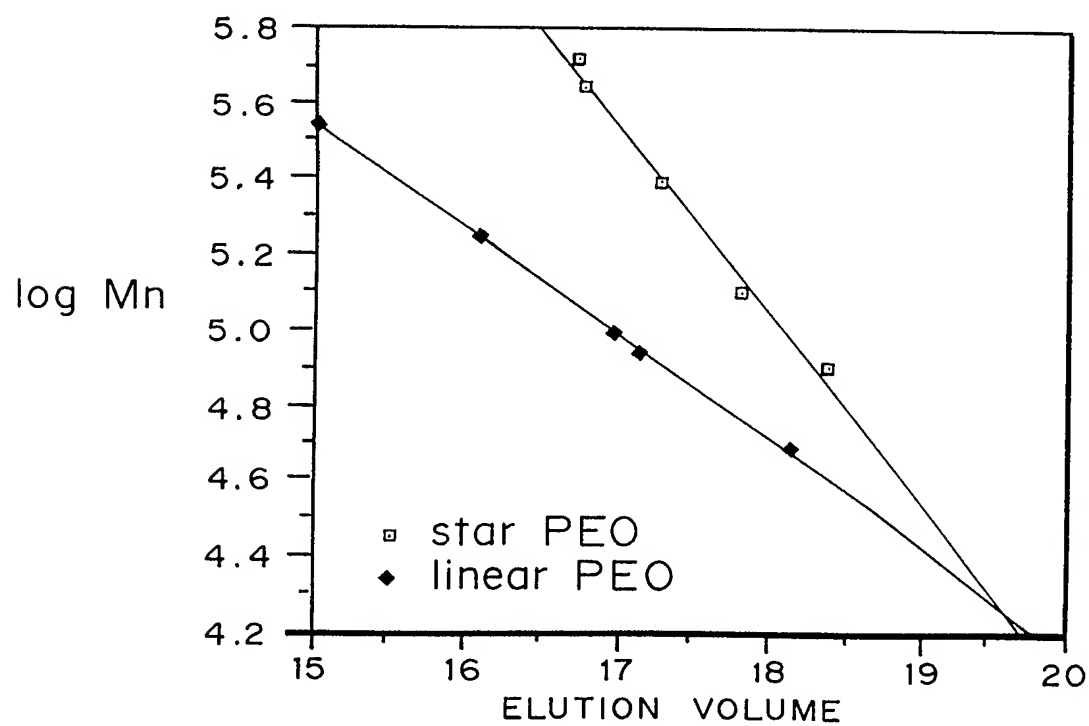


FIG. 13

SUBSTITUTE SHEET (RULE 26)

FIG. 14 PEO Type	MPEO Note a	Dendri- mer function- ality (fd)		Dendri- mer Mol Weight (Md)		Star Mn expected		Star Mn found		Star function- ality fs		Fraction of dendrimer functions used fs/fd
		Note b	Note b	Note b	Note b	Note c	Note d	Note d	Note d	Note e	Note e	
MeO-PEO-CH ₂ CH ₂ CONHS	5000	16	3206	83256	88700	1.09	17	1.06	17	1.06	1.06	
MeO-PEO-CH ₂ CH ₂ CONHS	5000	32	6909	166909	161000	1.06	31	1.06	31	0.97	0.97	
MeO-PEO-CH ₂ CH ₂ CONHS	5000	64	14215	334215	268000	1.01	51	1.01	51	0.80	0.80	
MeO-PEO-CH ₂ CH ₂ CONHS	5000	128	28000	668000	496000	1.06	94	1.06	94	0.73	0.73	
MeO-PEO-CH ₂ CH ₂ CONHS	5000	256	50845	1336000	778000	1.03	144	1.03	144	0.56	0.56	
HO-PEO-CH ₂ CH ₂ CONHS	4000	16	3206	67260	76600	1.15	18	1.15	18	1.38	1.38	
HO-PEO-CH ₂ CH ₂ CONHS	4000	32	6909	134909	121000	1.2	29	1.2	29	1.06	1.06	
HO-PEO-CH ₂ CH ₂ CONHS	4000	64	14215	270215	235000	1.24	56	1.24	56	1.03	1.03	
HO-PEO-CH ₂ CH ₂ CONHS	4000	128	28000	540000	416000	1.05	97	1.05	97	0.90	0.90	
HO-PEO-CH ₂ CH ₂ CONHS	4000	256	50845	1080000	498000	1.04	112	1.04	112	0.52	0.52	
t-boc-PEO-CH ₂ CH ₂ CONHS	3400	64	14215	231815	177000	1.05	48	1.05	48	0.81	0.81	

Notes:

(a) MPEO, MOLECULAR WEIGHT OF THE PEO, g-mol⁻¹, AS REPORTED BY SUPPLIER, SHEARWATER POLYMERS, INC.

(b) NUMBER OF AMINO FUNCTIONS, fd, AND MOLECULAR WEIGHT, Md, OF THE DENDRIMER AS REPORTED BY SUPPLIER, ALDRICH CHEMICAL CO. AS SELLING AGENT, OR DENDRITECH, INC.

(c) CALCULATE BY THE FORMULA: $Mn, ex = MPEO \cdot fd + Md$, WHERE Mn, ex = EXPECTED MOLECULAR WEIGHT OF THE PEO STAR MACROMOLECULE(d) DETERMINED BY GPC-LS, Mn, fd IS THE EXPERIMENTALLY DETERMINED MOLECULAR WEIGHT OF THE PEO STAR MACROMOLECULE. BY GPC-LS THE WEIGHT AVERAGE MOLECULAR WEIGHT MW IS ALSO DETERMINED, AND Mw/Mn IS THE POLYDISPERSITY INDEX pdi(e) APPARENT STAR FUNCTIONALITY, fs, AS CALCULATED BY THE FORMULA: $fs = (Mn, fd - Md) / MPEO$

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/19257

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/48 C08G65/32

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C08G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 171 264 A (E.W. MERRILL) 15 December 1992 cited in the application see column 2, line 44 - line 68 ---	29,40
X	WO 93 25247 A (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 23 December 1993 see page 12, line 24 - page 13, line 26 ---	29,40
X	Y. GNANOU ET AL.: "Synthesis of Star-shaped Poly(ethylene oxide)" MACROMOLECULAR CHEMISTRY, vol. 189, 1988, pages 2885-2892, XP002055826 cited in the application see page 2890, paragraph 2 - page 2892, paragraph 1 --- -/--	29,40

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

16 February 1998

Date of mailing of the international search report

04.03.98

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Boeker, R

INTERNATIONAL SEARCH REPORT

Intern. Application No

PCT/US 97/19257

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 461 116 A (R. HOXMEIER ET AL) 24 October 1995 see claims ---	1
A	E. W. MERRILL: "Poly(ethylene oxide) Star Molecules: Synthesis, Characterization, and Applications in Medicine and Biology" J. BIOMATER. SCI. POLYMER ED., vol. 5, no. 1/2, 1993, pages 1-11, XP002055827 cited in the application see the whole document ---	1-42
A	D. TOMALIA ET AL.: "Dendritic Macromolecules: Synthesis of Starburst Dendrimers" MACROMOLECULES, vol. 19, 1986, pages 2466-2468, XP002055828 cited in the application see the whole document ---	1-28
A	HARRIS J M: "LABORATORY SYNTHESIS OF POLYETHYLENE GLYCOL DERIVATIVES" JOURNAL OF MACROMOLECULAR SCIENCE-REVIEWS IN MACROMOLECULAR CHEMISTRY, vol. C-25, no. 3, 1985, pages 325-373, XP002036446 cited in the application see the whole document ---	1-42
A	VOIT B I ET AL: "DENDRITISCHE UND "HYPERBRANCHED" POLYMERE 1 BEISPIELE FUR DIE BEEINFLUSSUNG VON POLYMEREIGENSCHAFTEN DURCH GEZIELTE SYNTHESE VON DREIDIMENSIONALEN STRUKTUREN" ANGEWANDTE MAKROMOLEKULARE CHEMIE, vol. 223, 1 December 1994, pages 13-27, XP000482382 see page 24, paragraph 2 -----	1,29,40

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. Application No

PCT/US 97/19257

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5171264 A	15-12-92	AT 105735 T	15-06-94
		CA 2074730 A	29-08-91
		DE 69102062 D	23-06-94
		DE 69102062 T	05-01-95
		EP 0516749 A	09-12-92
		ES 2056641 T	01-10-94
		WO 9112886 A	05-09-91
		US 5275838 A	04-01-94

WO 9325247 A	23-12-93	US 5275838 A	04-01-94
		AU 4533893 A	04-01-94

US 5461116 A	24-10-95	CN 1129713 A	28-08-96
		EP 0711789 A	15-05-96
		JP 8208780 A	13-08-96
